Title

Polyketide Synthase Gene from *Sorangium cellulosum*

Cross-Reference to Related Applications

The present application is a continuation-in-part of allowed U.S. patent application Serial No. 09/144,085, filed 31 Aug. 1998, which is a continuation-in-part of U.S. Patent No. 6,090,601, each of which is incorporated herein by reference.

Field of the Invention

The present invention relates to the fields of molecular biology, chemistry, and medicine.

Background of the Invention

Sorangium species produce a variety of useful polyketides, including epothilone, myxothiazole, and soraphen. U.S. Patent Nos. 5,962,290; 6,066,721; and PCT patent publication Nos. 98/49315; 99/0398600/24907; 00/31247; and 00/44717 describe methods for making novel polyketides by combining portions of two or more polyketide synthase (PKS) genes to create novel genes that encode a hybrid PKS and by providing synthetic biosynthesis intermediates to modified PKS enzymes. There remains a need for new polyketide synthase genes for use in the preparation of hybrid PKS enzymes and the polyketides produced by such hybrid enzymes. The present invention meets that need by providing recombinant DNA compounds that comprise all or a portion of a PKS gene from *Sorangium cellulosum*.

Summary of the Invention

The present invention provides recombinant DNA vectors and host cells that comprise the *tmbA* genes of *Sorangium cellulosum* or fragments of those genes.

These and other aspects of the invention are described in more detail in the following description and claims set forth below.

Brief Description of the Figures

Figure 1 provides a physical map of the *tmbA* gene cluster and an alignment of the cosmids of the invention (34-7, 28-26, and 14H12) that comprise the *tmbA* gene cluster genes and gene fragments. The PKS genes are designated *tmbA*, *tmbB*, and *tmbC*. Open reading frames (ORFs) are designated 1 through 8, inclusive. ORFs 1 and 3 are overlapping. ORF 1 is a thioesterase (TE); ORF 3 is a methyltransferase. ORF 4 is a hydrolase. ORF 6 is an epoxide hydrolase.

Figure 2 provides a structure of the polyketide tombamycin produced by the TmbA PKS in monomeric and dimeric form. R is a is a substituted or unsubstitued C_3-C_8 alkyl or cyclic alkyl.

Detailed Description of the Invention

The present invention provides recombinant DNA vectors that comprise all or a portion of any of the genes in the *tmbA* gene cluster. The tmbA gene cluster is comprised of PKS genes *tmbA*, *tmbB*, and *tmbC*, and ORFs 1 - 8, inclusive. Each PKS gene in the cluster is composed of one or more PKS modules, each comprising an acyltransferase (AT), ketosynthase (KS), and acyl carrier

protein (ACP) domains and optionally one or more ketoreductase (KR), dehydratase (DH), and enoylreductase (ER) domains as well as linkers that connect one domain to another and one module to another. The boundaries of each of these domains can be identified by sequence comparison with known PKS genes and enzymes. In one important embodiment, the invention provides recombinant DNA vectors that encode all or a portion of one or more of these domains that are useful in the construction of hybrid PKS genes and enzymes.

The sequence of the *tmbA* gene cluster is shown below.

CTCCAGATCGACCTGCATGATCTTGCCGACGAGCTGCAGCAGCTCAGGGTCCTCCTGGAT
CACACGATCGAACGCCCCGTACCTCTGCTGTACACGCCGCAGGAACTCGCTCGCGGGGAG
GTGAGAGATCGGCGGCAGGCGGGCGGGTACGTGAGGGGCTCTGCTCGCGGCGACGACCAT
GCATGTCGGCTCGATCCCACGGCGCCTGCGGAGAGAACGGGCCCATTCGAAGGCCATCAG
CGCTCCCAGGCTGTATCCAAAACACGCGAACGGCAGGTCGAGCATGTCATCCAGCCCCCG
CTCGATGCCGTCGATGAACTCCGACATCGAGCGAGGTTGCTTCTCCTTGGACCGAGCGAA
TCGCCCCGGAGGCTCTATCGGGCAGACGTCGATATGCGCCGGTAGGCTCTTCGCCCAGTC
TCGATATATGGCCCCGCCGGCGCCGGCGTAGGGGAAGCAGAAAAGGCGAAGCTTCGCGTC
CGTCCGCGCGGTCCAGTGCGACAGCCAGGGATTCTTCTCCATGTAGACCTCCGGTGCAAG
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CGCGTCAACCGGCTCCGTGAGAGGCGATACGACCTCACGGCGAAGGCGGCATCCCGATGC
CGGCGCCACGACCAATGGGGCGCCGATTCTATCCATCCAGCCTCGCACACGATTTCGATG
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AGACATCGCCATGAACACGCGAAAAATTCGTATCGTACTGTATGCCGCGCTCACGCTGAT
CGGCGCTGCCGGCGTTTTGTGGTTCAATATACGGTTCATGAACGCCGGGGGGTTCGATTC
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CGAGGACGCAGGAACGGTAGCGTCCGACGTTGACGATCACCTGCACGACCGCGCTCACGA
ACAGGCTCGCAGTGAGCATGATCCCTTTAGTGAGGGTTAATTGCGGCCGCC

The sequence of each of the ORFs in this gene cluster and the translated amino acid sequence of the proteins encoded thereby are shown below.

orf1 partial sequence bases 522-1

ATGGAGAAGAATCCCTGGCTGTCGCACTGGACCGCGCGGACGGACGCGAAGCTTCGCCTT
TTCTGCTTCCCCTACGCCGGCGCCGGCGGGGCCATATATCGAGACTGGGCGAAGAGCCTA
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CTGCTGCAGCTCGTCGGCAAGATCATGCAGGTCGATCTG

MEKNPWLSHWTARTDAKLRLFCFPYAGAGGAIYRDWAKSLPAHIDVCPIEPPGRFARSKE
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orf2a bases 791-1144

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orf2b bases 1233-760
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orf3 bases 2171-1230

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SGEKPGNMPHSAR

orf 4 bases 3456-2248
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tmbA bases 3853-31557

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TmbB

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RRGVR

orf5a

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TGA

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orf5b
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RTSECVPSRWLMTKSPVVT

orf 6
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orf 7
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G

orf 8 - partial sequence
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ARERAVAKPS

The genes of the *tmbA* gene cluster can be isolated from the cosmids of the invention shown in Figure 1 or from *Sorangium cellulosum* genomic DNA.

The gene products of the *tmbA* cluster can be used to synthesize the polyketide tombamycin, the structure of which is shown in Figure 2. Tombamycin can be dimerized to produce the dimeric form of tombamycin, the structure of which is also shown in Figure 2.

The invention having now been described by way of written description those of skill in the art will recognize that the invention can be practiced in a variety of embodiments and that the foregoing description and examples are for purposes of illustration and not limitation of the following claims.